THE MÜTTER LECTURES ON SELECTED TOPICS IN SURGICAL PATHOLOGY.

SERIES OF 1890-1.[1]

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LECTURE V.

PERITONITIS.—TESTS FOR ANTISEPTICS.

SYLLABUS.—*Peritonitis.* The peritoneum and its capability of absorption; rapidity of same. Influence of any ascitic fluid present. Effect of injections of various pyogenic organisms and in varying quantities. Infection of peritoneal wounds. Forms of peritonitis. Conditions under which infection takes place Improbability of a pure type of gonorrhœal peritonitis. Distinction between septic and putrid forms of peritoneal inflammation.

Testing antiseptics. Method of testing the relative antiseptic value of a chemical substance with various pathogenic organisms. By the hanging drop. With spore-threads. Determination of necessary length of exposure. Results with solid culture media. Estimate of its absolute as well as its relative toxicity.

Application of these methods to an estimation of the new drug *Pyoktanin* Disappointment experienced here as with all other drugs of its class.

OUR views concerning the susceptibility of the peritoneum and its intolerance of insult have undergone wide changes within the past few years; indeed it would almost seem to be more tolerant than other serous cavities or ordinary subcutaneous tissues. The explanation of this condition is to be found in the character of this membrane, and the conditions which obtain when pyogenic organisms are therein introduced; and first of all comes into play its wonderful capability of ab-

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sorption, by which micro-organisms are deprived of their necessary nutrient fluid, along with which, as it returns to the vessels, it is quite possible they may pass into circulation to be there destroyed. For instance, Wegner introduced 200 cc. of warm serum into the peritoneal cavity of a rabbit, and an hour later bled the animal to death; the amount of fluid then present in the same cavity was only 66 cc. showing that 134 cc. had been absorbed within an hour. When fluid of lower specific gravity than blood serum is introduced, it seems to lead at first to a transudation from the blood. It is very different with a fresh wound, since here we have not an active absorbing surface, but rather the contrary. Wegner further showed that a great variety of fluids free from bacteria, such as water, urine, bile and blood may be introduced in the same way without bad results. So even with large quantities of unfiltered air. If too large a quantity of fluid capable of putrefaction be introduced at the same time with unfiltered air then there is rapid decomposition. Thus if 50 cc. of such fluid are introduced into the abdomen of a rabbit, of which only a part can be rapidly taken up, the rest furnishes a suitable medium for the growth of the organisms present in the unfiltered air which has been injected, inasmuch as such air contains ordinarily saprophytic but not pyogenic organisms. Wegner more often produced septic intoxication than true septic peritonitis.

Reichel has quite recently published an essay containing a mass of clinical reports, in which he seeks to discover what particular conditions favor the occurence of peritonitis. A series of experiments in which he injected pus with gelatine into the peritoneal cavity confirm the statement of Wegner and of Grawitz that the peritoneum is capable of absorbing a certain amount of pyogenic organisms, but that injections of relatively too large amounts are fatal. He made a number of experiments by excising a small area of peritoneum and rubbing into its denuded surface the organisms with which he was experimenting. Four out of five animals withstood this inoculation, although Grawitz did not meet with the same success in similar experiments. The results gathered from a large number of abdominal sections in human beings agree

pretty well with his experimental results. In complicated ovariotomies the peritoneum equally easily reabsorbs these foreign organisms. Ascites, which frequently occurs, offers a fine culture fluid for the growth of bacteria, and increases the danger of peritonitis. Separation of adhesions favors inflammation just as in the experiments above detailed, and for the same reason. A recent or fresh attack in infectious peritonitis does not seem to increase the danger of septic inflammation. (*Deutsche Zeit. f. Chir.*, xxx, 1 and 2).

Predoehl has investigated fourteen cases of suppurative peritonitis. The streptococci were found most commonly.

Fraenkel bas also assigned the predominating rnle in peritonitis to the streptococci, finding them in two-tbirds of all cases, especially in the more rapid forms. Only in the more slow cases did other forms appear, which proceeded prohably from the intestine, and seemed sometimes to destroy the streptococci or at least antagonize their action. Fraenkel cultivated the streptococcus pyogenes from this source, and with it produced a typical erysipelas on the ear of a rabbit; also a typical peritonitis and panopbthalmitis after injecting it respectively into the peritoneum and the eye.

Pawlowski undertook a series of experiments to cornborate the statements of Grawitz, who injected large quantities of staphylncoccus aureus into tbe peritoneal cavity, without producing peritonitis.. He had already shown that after the injection of such irritating substances as crnton oil, an inflammation of a sero-hæmorrbagic character, but not septic, was prnduced. He began this latter series with relatively large quantities, which he gradually reduced, and found tbat only small quantities were absorbed without injury. With the bacillus pyncyaneus he produced for the most part only a fibrinous hæmorrhagic inflammation, which several days later became purulent. He also endeavored to ascertain just what element of the intestinal cnntents produces perforation peritonitis. He showed that filtrated intestinal contents free from hacteria are not pathogenic. Evidently, therefore, hacteria are the agents, and he isolated a sbort, rapidly growing bacillus, the injection of which into the peritoneum produced suppuration for a time. He also sbowed that given quantities of staphylococci whizh by themselves were incapable of causing inflammation, could, nevertheless, do so if croton oil were introduced at the same time. (*Virchow's Arch.*, 117).

In opposition to Pawlowski, Waterhouse, working under the direction of Orth, came to results which agree for the most part with those of Grawitz. He was able to inject much larger quantities of staphylococci into the peritoneum, it making no difference whether they were injected through a canula, or after incision into the peritoneum with the endeavor not to injure the intestine. He seems to have demonstrated that

peritonitis occurs only when absorption is interfered with. He found also that the simultaneous introduction of blood, or strong meat broths, or ammoniacal urine or turpentine, along with the introduction of pus is always dangerous So far as the infection of peritoneal wounds is concerned, it appears from his experiments that a well sutured wound does not favor growth of organisms, but that large defects in the abdominal wall, or the mesentery, prove favorable fields for the growth of bacteria. Previous disease of the peritoneum, such as ascites, seems to favor peritonitis. He found that after injecting staphylococci several hours after an artificial obstruction, which latter of itself would be harmless, purulent inflammation supervened. Especially noteworthy were his results when, after producing such intestinal obstructions, he injected the cocci, not into the peritoneal cavity, but into the veins, or into the bones, muscles, or other tissues. It appears therefrom that the microbes thus brought into contact with the intestinal lesion do not perforate intestinal walls as long as they are not necrotic. (*Virchow's Arch.*, 119).

At the sixty-second Congress of German Naturalists, Orth detailed some of his experiments in the production of peritonitis. Alluding to the wonderful resistance which the peritoneums of animals have shown after injections of putrid substances, etc., he claimed that if the peritoneum be injected with the same material in less absorbable shape the result is different. Such a condition is obtained when pure gelatine and agar cultures of pyogenic organisms are used, or fifteen to twenty centimeters of freshly injected blood, so that some remnant of the injection can still be found three days later. He thinks that large amounts of pure blood clot alone are sufficient to set up peritonitis, probably not alone from bacteria but from some fibrin ferment.

Previous visceral lesions favor the activity of these organisms. In ascitic animals 1 cm. of staphylococcus fluid will cause fatal results in three to four days. In the same way excision of a piece of peritoneum, or irritating a small area with turpentine, lower its resistance greatly. Numerous experiments were made to estimate the effect of vascular disturbances. Loops of intestine were strangulated for various times. It was found that a ligature of the loop for four to six hours, by itself, did no harm, but if this were followed by injection of the above fluid peritonitis quickly

ended the animal's life. Even when the strangulation lasted hut two to four hours, the injection of four drops of fluid into the vein of an ear caused death in twenty-four hours. The streptococci appeared a little more slow in their action than the staphylococci.

If after temporary artificial strangulation a fracture be subcutaneously made, and the injection made into the fracture wound, the result is the same. In other words, by indirect or direct introduction of pyogenic organisms peritonits can be set up and rapidly prove fatal *providing* the local disposition be present.

Grawitz, in a careful study concerning the origin of peritonitis, has formulated the following dictum.

Pyogenic organisms mixed with absorbable amounts of indifferent fluid and injected into the normal peritoneal cavity produce peritonitis only when:

1. Introduced in excessive amount.

2. When at the same time some substance acts to produce necrosis of the tissue and thereby to prepare the way for penetration of the cocci into the deeper layers of the serous membrane.

3. When especially some wound of the abdominal wall favors localization of infection. (*Charite Annalen.*, 1886).

Pawlowski, in his researches elsewhere alluded to, had some curious results bearing on the topic now under consideration. He found that injections of fresh normal fæces gave rise to fatal fibrino-purulent peritonitis, which he considered to be produced by a particular bacillus which he termed *bacillus peritonitis ex iutestinis cuniculi.*

He agrees with observers who claim that the pyogenic cocci all thrive with the greatest activity when they find in the peritoneal cavity any dead or dying tissue or cells.

He distinguishes two forms of peritonitis:

1. That produced by chemical agencies such as croton oil and trypsin, of hæmorrhagic form.

2. That produced by infection.

a. Peritonitis mykotica, of violent severity, without peculiar macroscopic features, showing microscopically exuberant proliferation of the micro-organisms on the serous surfaces.

b. Less violent type, beginning as a hæmorrhagic form.

c. Fibrino-purulent form, representing the mildest infectious variety; usually the commencement of the ordinary purulent peritonitis.

In general, Bumm makes the following different classification of forms of peritonitis, yet one which is certainly accurately founded.

I. Aseptic, usually local, sometimes generalized. It progresses to fibrinous exudate and possible adhesions. In this form there is no bacterial invasion.

II. Septic.

a. Streptococcus and staphylococcus peritonitis. Usually puerperal.

b. Putrid peritonitis. Usually post-operative or perforative. Is a mixed infection. (*Vide* Lect. X.).

The former happens most often after parturition, begins with a chill, and is accompanied throughout by high fever. On section is found thin, purulent, odorless exudate, or if late this may be thick and creamy; this exudate early in the disease is very infectious, but loses in virulent intensity as the disease progresses. As the streptococcus belongs to the facultative aerobic organisms and loses its virulence by exposure to the air, we may find here the explanation of the fact that the exudate is more infectious than the cultures of the germ. The path of infection from the genitals to the peritoneum is by no means always clear, since sometimes the tubes are quite free from the organism.

The putrid peritonitis occurs most commonly after operations; it begins without chill, with fever, which gradually runs higher, and is characterized by a putrid, ill-smelling exudate. This is slightly, if at all infectious, and contains a mixture of several organisms, many of which at least are in no wise pathogenic. This disease is the result of putrefactive organisms, which extend at the time of the operation, and quickly work their evil effects. The febrile symptoms are mainly due to ptomaines. The disease is spread locally by movements of the bowels, peristalsis. Other forms of mixed infection from perforation, etc., can hardly be classified.

III. Specific. Tubercular; Gonorrhœal (?). This last form

is as yet problematical. (*Münch. med. Wochenschrift*, 1889, No. 42).

Bumm further questions the possibility of a pure type of gonorrhœal peritonitis. Pure gonorrhœal pus which escapes into the peritoneum from an infected tube-sack, acts, he says, like an aseptic foreign body, and becomes encapsulated. If the contents of the tube present a mixed infection then the result may be very different.

Pernice experimented extensively to help settle this operation. He found that various chemicals like concentrated acids, phenol, strong corrosive sublimate solutions, etc., injected into the abdomen of guinea-pigs and rabbits, produced undoubted peritonitis with necrosis and perforation. But the character of the exudate was always serous or sero-fibrous, never purulent. (*Rivista Inter. di med. e. Chir.*, 1887).

Pawlowski made over a hundred quite similar experiments. Croton oil and trypsin in dogs and rabbits produced acute bæmorrhagic but not purulent peritonitis. Non-pathogenic organisms were introduced in large numbers; they produced no inflammation, even when introduced with small doses of irritating chemicals. Quite otherwise, with cultures of the pyogenic microbes; the staphylococcus pyogenes aureus produced frequently fatal purulent peritonitis; indeed he had much more pronounced results with these cocci than some others have had.

The ordinary septic peritonitis following confinement is a streptococcus infection Whether the cocci work along through the vaginal and uterine surfaces and through the tubes to the peritoneum, or whether they pass by the lymphatic vessels directly to the serous covering of the uterus, has not yet been definitely settled; if indeed they do not take either course according to circumstances. In two cases of this nature, however, Bumm has found the tubes completely free of bacteria.

In the most rapidly fatal cases one finds in the peritoneal cavity a thin, flaky, yellowish fluid, which if removed by aspiration immediately after death has no odor. It contains fibrin flakes, endothelial and pus cells, and streptococci. These latter are found as well in the genital tract, in the blood and most of the internal organs. This fluid is extremely infectious. A fraction of a drop injected into the abdomen of a rabbit sets up a violent commotion which is fatal in twenty-four hours. Injected in the tissues in trifling amount and well diluted it sets up an acute phlegmon which is rapidly fatal.

In the slower forms of puerperal streptococcus-peritonitis the peritoneal exudate is more purulent in appearance, but less virulent in its properties. Of this it takes from a few drops to two grammes to set up a fatal peritonitis in a rabbit.

So also the reaction on subcutaneous inoculation is less violent. It appears, as Bumm says, as if the virulence of the material is the more diminished the longer it is exposed to the action of living cells.

Experiments made with this exudate from fresh cases give constant results; it seems however that experiments made with pure cultures of the same organisms are followed by most uncertain consequences. It is hence abundantly proved that by cultivation these organisms loose their virulence, a fact which we know as well of the bacilli of anthrax and tubercle. Widal has referred this peculiar alteration of malignity to the fact that these cocci when at their best are ærobic, and that when cultivated in a hydrogen atmosphere they retain their infectiousness. The fact well known to laboratory workers that streptococci grow better along the needle streak than on the surface also bears out this view.

The course of an ordinary traumatic (post-operative) peritonitis, like the findings, is somewhat different. The dirty looking, sometimes badly smelling, peritoneal exudate contains now not any specific organism, but shows a mixed infection, cocci and bacilli being often found together. By plate cultures several different forms can be isolated. Intra-abdominal injections of such cultures, in rabbits, usually give no results. Only the original peritoneal exudate, and this often in considerable quantities, seems not to be infectious.

Between these two varieties of peritoneal inflammation there are thus seen to be differences not merely clinical; and Bumm, as shown above, has proposed to call the former the *septic*, the latter the *putrid.* His explanation is about as follows: No one who has done bacteriological work but knows full well that no laparotomy can be done without exposure to germs and their contact with the parts exposed. Aseptic operating comprises, virtually, exclusion of the majority of organisms and trusting to the resistance of the tissues to dispose of those not excluded. Ordinarily such microbes as enter the abdomen are killed by the cells or fluids in which they lodge. But when the peritoneum is too severely attacked, or is already weakened in resistance, then surroundings are made favorable for such germs as have entered, and the process if once begun

can scarcely be checked. By peristaltic action infection is spread, and by the peculiar capability of absorption which the peritoneum possesses an enormous number of organisms enter the blood, so that patients soon succumb to putrid intoxication.

Between the septic and putrid varieties we have these distinctions:

In the former we have pus instead of ichor, and acute onset with chill and high temperature instead of a more deliberately and gradually febrile clinical picture.

As a result of these, and hundreds of similar experiments, we have learned that to produce suppurative peritonitis it is necessary either to introduce the cocci in such numbers along with their products, that a part of the peritoneum be so affected as not to exercise its proper function; or they must be introduced into an already unhealthy peritoneum, or there must be present too large a quantity of fluid to be quickly absorbed; or finally there must be present some material such as blood-clot, or dying or dead tissue, in which they can develop. (Cheyne) As Cheyne has shown, suppurative peritonitis occurs with the greatest certainty when there is a wound in the abdominal wall in which infection can occur, and from which, as a center, organisms are constantly given off into the cavity within. This is still more certain to occur if the wound be an unhealthy one. For example in rupture of a healthy bowel, if the extravasated contents are thoroughly removed and the wound early approximated, recovery commonly occurs. But in perforation after typhoid the bowel-wall is unhealthy and forms a nidus in which organisms may grow and the only prospect of success is by resection, that is removal of the unhealthy tissue.

E. Frankel has called attention to the clinical fact that the more rapid the case of peritonitis the more likely we are to find pure cultures of the streptococcus in the pus. (This can hardly apply to a perforative form of the disease.) He also shows how hard it is to always recognize streptococci on gelatine cultures alone and at ordinary temperatures, and how much more accurately this may be done with glycerine-agar media at blood temperature; and he ascribes, and with propriety, some of the negative or contradictory findings of pre-

vious observers to lack of this precaution. He also regards this streptococcus (pyogenes) as identical with that of erysipelas; and he has produced this latter disease by inoculating animals with pure cultures taken from the abdominal cavities of animals with peritonitis. With the same organisms injected into the eye he has produced, moreover, panophthalmitis, and when injected into the cellular tissue purulent infiltration.

Of the many non-specific organisms often met with along with the streptococcus, most possess the property of curdling milk and of decomposing albumen, and this latter property certainly works no benefit for the patient. Many of them produce ptomaines which have highly toxic properties. Boiled cultures of these organisms are still highly toxic, which is not true of streptococcus.

Fraenkel can hardly agree with Bumm in his differentiation between *septic* and *putrid* peritonitis, but he finds that the pure streptococcus forms give at least an odorless exudate.

He further describes a form of peritonitis determined by inorganic chemical agencies, and alludes to the frequency with which gynæcologists use tincture of iodine and iron salts. These substances, even when absolutely sterile, have the power of provoking a sero-fibrinous but not purulent inflammatory exudate, which is absolutely free from organisms and odorless. If the animal or patient live long enough this may be invaded by organisms from the intestinal canal. (*Münchener med. Woch.*, 1890, No. 2, p. 23).

The Testing of Antiseptics.

For the purpose of testing an antiseptic it is not enough to mix it in certain definite proportions with various nutrient media, and then endeavor to ascertain whether this or that organism will grow therein. Even if it will thus grow we have still very much to determine as to matters to be commented upon later; whereas, if it will not grow upon one or two trials it might be assigned an altogether false position.

There is systematically carried out in the laboratory of the Hygenic Institute in Berlin a method which, though long and somewhat tedious, leaves virtually nothing to be desired in determining the exact bactericidal properties and toxic effects of a given agent. It is practically a method laid down by the great master Koch, and carried out and taught by his assist-

ants, to whom, especially to Dr. Behring, I am indebted for an acquaintance with it. It is briefly as follows: A soluble antiseptic must be dissolved in solutions of known strength; an insoluble material can hardly be properly tested. We begin, therefore, with a standard solution of the substance to be tested, and this should be of the strength of, say, 1 to 1,000. It is now convenient, knowing the dropping glass or the pipette with which we are to work, to ascertain now many drops, as they fall from its point, will constitute 1 cc. Let us suppose for illustration that this number is 80; obviously then, one drop of this standard solution contains $^1/_{80,000}$ of a gram of the substance to be tested. Two drops equal $^1/_{40000}$ four drops equal $^1/_{20000}$, and forty drops then equal $^1/_{2000}$. We experiment first with bouillon duly sterilized, and in sterilized tubes. It is best also to select three typical pathogenic organisms with which we shall conduct three preliminary series of experiments.

First, anthrax, which is the most resistant and tolerant of all of the common forms; and second and third, the staphylococcus aureus and the streptococcus pyogenes, which are representative species of generic groups that give surgeons the greatest trouble. Now 4 cc. of sterilized bouillon are placed in a tube and inoculted with a fresh, pure culture of anthrax. After the tube is thoroughly shaken, a small drop of the infected bouillon is removed with a fine platinum loop, placed upon a clean cover-glass, and this is inverted over a hollow slide, and sealed with vaseline; in other words, this is a pure culture of anthrax in a hanging drop, and is used for control. To the same tube of bouillon is next added one drop of the standard solution above referred to. One drop mixed with 4cc. now gives to the solution a strength of $^1/_{320000}$. This is shaken and a drop of this placed upon another cover-glass. A second drop is now added to the same tube, which so far strengthens the solution as to give it now a strength of $^1/_{160,000}$; after making a culture of this strength, two drops more are added, thus making it $^1/_{80000}$. The next dilution is made with four drops more, which, with the four previously added, make eight drops in all, or a strength of $^1/_{40000}$. Next, eight drops more are added, giving it a strength of $^1/_{20000}$, and next sixteen

more, which with the previous sixteen, make thirty-two drops now added to the solution, and giving it a strength of $^{1}/_{10000}$. This process is carried out as far as we choose to conduct it, making a fresh hanging drop culture with each fresh addition of standard solution. Each slide is carefully marked with the character of the culture and the strength of the solution, and all are the placed in a cage or suitable holder, which is then placed in the thermostat where it is kept at blood heat; after twenty-four hours the slides are removed and each one carefully examined under an immersion lens. A table is then constructed showing in just what strengths of solution bacteria are found after this interval, where they begin to fail, and where they are not found. The slides are then restored to the oven and the same observations are reported at the end of the second and of the third day. The results thus obtained give us our first working data with the organism in question.

In the experiments which we are supposing, the same investigations must be made with the other two forms of bacteria above alluded to, since it will be found that a solution strong enough to kill staphylococci will by no means necessarily destroy the anthrax bacilli.

Conversely, however, we may hold that anything which will destory anthrax bacilli will almost certainly kill all other pathogenic bacteria.

Next, we introduce a series of cultures made with the so-called spore threads. These consist of ordinary linen or cotton threads which have been sterilized by heat, and which are then left for a few hours in pure bouillon or fluid cultures of the above organisms; they are then removed and dried in a safe place. With organisms which produce spores these threads become impregnated with the same, and the latter will preserve their vitality for months or even years. If, now, small particles of these threads be clipped off with sterilized scissors, and a little particle immersed in our hanging drop, there will develop there the typical organism just as under other favorable circumstances. These spore threads are used in much the same way, as above detailed; a control culture is first made, or better two of them, by placing one of these particles in a hanging drop of pure bouillon. To 4 cc. of this same

bouillon are now added, drop by drop as before, given amounts of the standard solution; with the addition of each proportion a hanging drop culture being made, by inserting a particle of spore thread.

By means of the first series of experiments just detailed, we can narrow down within certain limits the proportions between which we must further work, and our experiments are thus made less discursive. These spore thread cultures are placed in the thermostat as before, and observations are made at the end of the first, the second and the third day, and the results tabulated again. This method is simple, and has much in it to attract and commend itself. It is, however, open to serious error, inasmuch as the various antiseptic solutions exert different effects upon the material of the thread or are themselves altered by it. For instance, if it is with aniline dyes that we are experimenting, the vegetable fibres take up a certain amount of coloring matter, thereby depriving the solution of so much, and vitiating our calculations. If it is with such substances as mercuric chloride, zinc chloride or silver nitrate that we are working, they also undergo mutual reactions with the same disturbance of relative strengths. So that before these tests can be considered thoroughly reliable, we must determine what these mutual reactions are. There is, further, a most important practical deduction from the above statements, since for wound dressings we depend upon vegetable material, usually cotton, which is saturated or impregnated with antiseptic solutions of various strengths. It will be seen, therefore, that it does not follow that by the time these dressings are acted upon by wound discharges, the proportion of antiseptic which they contain will be the same as at first prepared; in other words, a so-called antiseptic dressing may not be nearly as much of a protection as it would appear to be.

After determining the value of an antiseptic by the hanging drop experiments, it is necessary to determine its activity in the direction of the length of exposure necessary for the destruction of bacteria by solutions which have a sufficient strength, as determined above, to produce a bactericidal effect. For instance, in a strength of $^1/_{3000}$ a given organism does not grow in the hanging drop after 24 to 72 hours. If this has

been determined, we must next make clear how long it takes a solution of this strength to kill this same organism. Suppose that we are working with a given antiseptic designated by X, and with anthrax.

Bouillon is impregnated with this X in a proportion of one to three thousand, it is then inoculated with a pure, fresh culture of anthrax, and carefully shaken. At stated intervals one drop is taken from this tube and planted in another of pure bouillon; these tubes are then placed in a thermostat and, after 24 hours' exposure there, the results as to growth or no growth are carefully noted. The intervals alluded are purely arbitrary, but are as follows: After five minutes, after two hours, and after twenty-four hours. The first period of five minutes is selected as representing such exposure as the conventional irrigation of a wound would offer; and the second and third are purely matters of convenience. These experiments should be repeated, only using blood-serum instead of bouillon. Such experiments have the definite object of demonstrating whether the given antiseptic, X, is of value when used as irrigating fluids are usually used in surgery; and they must be repeated with the staphylococcus and the streptococcus. Then bouillon cultures made like those just referred to should be mixed with X in the same proportion, and after the same intervals of time should be injected into animals, and results noted.

Furthermore, it is necessary to determine whether after a given time, say five minutes, all the organisms in a given tube are killed, or only the larger proportion. For these purposes take 5 cc. of pure bouillon in a tube, inoculate it with anthrax, shake thoroughly, remove 0.1 cc. with a sterilized pipette, add this to 5 cc. of gelatine, and make a plate culture in which after twenty-four hours the colonies are to be counted. Into the same tube of bouillon put X in the proportion of 1 to 3,000, and after five minutes again remove 0.1 cc. with the pipette, add this to 5 cc. of gelatine, make another plate culture, and so again after two hours and after twenty-four hours. After one day's exposure of these plates, which are supposed to have been kept at the same temperature with the same surroundings, either in a room or in a thermostat where the temperature is somewhat low, a count of each plate

is made. The number of colonies in the first plate, multiplied by 50 (=5 x 0.1), represents the number of bacteria in the tube of the bouillon before its inoculation; while the results gained from the other plates, multiplied by 50, show the various inhibitory effects of varying lengths of exposure. These experiments must be several times repeated, or several series must be undertaken at the same time, in order to give reliable data.

After determining the antiseptic power of a substance as the above investigation will reveal it, it is very necessary to determine whether it is poisonous or not. This is determined as follows: A given substance, for example X again, has been found to possess antiseptic, *i. e.*, bactericidal virtues in a proportion of 1 to 1,000. A rabbit weighing a thousand grams, as the average rabbit will weigh, or thereabouts, has injected subcutaneously one gram, in solution, of this same X; into another rabbit another gram is injected into the peritoneal cavity, while it is injected into a third by the intra-venous method. Each rabbit has now received $^1/_{1000}$ of its weight of X, and it remains to be seen whether the living animal can survive this strength any better than could the bacteria. If not, then X is to be considered *toxic*, and its *relative* toxicity is to be determined by further experiments conducted after the same fashion. If it can, then we have at last found that long desired substance which is parasiticide to bacteria, but with which the living organism can be impregnated in strength sufficient to kill such bacteria as may affect it.

But supposing that one gram of X is soluble only in 10 cc. of water, then our experimental rabbits must receive injections which are of themselves copious enough to injure or to kill. No rabbit can withstand the introduction at one time into the peritoneal cavity of 10 cc. of fluid. In such a case we take a smaller animal, for instance a white mouse, one such as will usually weigh 20 grams. This mouse must receive an intraperitoneal injection of $^1/_{1000} \div 20 = {}^1/_{50} = 0.02$ gr. This amount of the same X would equal $^1/_5$ cc. of fluid, which a mouse should easily bear in the peritoneal cavity. This method is, however, accompanied by difficulties. If we are experimenting with a strong antiseptic like a mercuric chloride, it can only be used in very weak solution, the strongest of which can

be used only in $^1/_{500}$ strength, otherwise it would act as an irritant or even caustic, and so prevent the results we desire to obtain.

This determination of the poisonous properties of X is essential if we desire to so saturate the system with the substance that its antiseptic properties shall be exerted throughout the body, and this method of determination must be carried out with great nicety. According to Behring, we must make out, not only the relative, but the *absolute toxicity* of a given substance; its absolute toxicity being the proportion in which it will kill an animal, its relative toxicity the proportion in which it will kill bacteria.

Until the present time no substance has been discovered whose absolute toxicity is not greater than its relative. In other words, we have not yet discovered that which will not kill in the animal in $^1/_4$ or $^1/_5$ of the proportion required to kill bacteria. When we have discovered that one of which this cannot be said, we shall have learned to conquer sepsis. In many respects the serum of certain animals most nearly approaches this desired substance, but this only for certain bacteria. It is, for example, known that anthrax bacillus will not grow upon rat-blood serum, although it will upon serum from other animals. Streptococci will not grow on calves-blood serum—only on rabbit-blood. Within the past few years numerous investigations have been made regarding the antiseptic properties enjoyed by blood serum, from which it would appear that it affords the greatest protection which our systems enjoy to have circulating in our blood serum of this healthy character. To discuss this matter would lead us too far from the subject in hand, and is a matter to be followed out upon some other occasion.

Studies like these were begun ten years ago by Koch, who himself carefully tested some two hundred different substances. He then turned over the work to Behring, who has investigated half as many more. Only very recently has any statement emanated from the master or his assistants indicating that any such substance had been discovered. Recent utterances of Koch imply that he thinks he has at last found it, at least so far as animals are concerned, and he there publicly

announced that he was ready to begin experiments with patients. His results remain to be heard. If he has been as careful and reliable in this work as in everything else which he has undertaken, we are on the eve of a fresh era in therapeutics.

The writer wishes here to express his personal indebtedness to Dr. Behring, of the Hygienic Institute in Berlin, as well as to his brochure "Ueber die Bestimmung des antiseptischen Werthes chemischer Präparate," etc. *Deutsche med. Woch.*, 1889, Nos. 41, 42, 43.

Experiments With Pyoktanin.

Applying now this method to present purposes permit me to report some investigations which I made last summer relative to one of the most recent candidates for bactericidal notoriety. During the meeting of the German Congress of Surgeons, (1890) there was exhibited by the Darmstadt house of Merck, a new antiseptic for which such claims were made as to stamp it—allowing for their truth—as a most important addition to the already large list. It was acknowledged and advertised to be an aniline derivative, but beyond this, at that time, nothing was told us of its *constitution* and its fanciful name, which had been protected, was calculated to reveal nothing. The improbable claim was made for it that it was capable of healing existing inflammations, and especially in wounds and ulcers. Also that it was perfectly innocuous, while its bactericidal properties were lauded as excelling those of sublimate. Along with circulars extolling its worth were sent out the brochure of J. Stilling, entitled *Anilin-farbstoffe als Antiseptica*, published just before the Congress. Something of his views may also be gathered from the following statements taken from a paper by Stilling, published in *Merck's Bulletin* (N. Y.) of June, 1890:

I have discovered that certain groups of colorific *coal-tar derivatives* possess all the properties to be demanded of *a really good medicinal disinfectant*, which shall not alone prevent infection, but which must also be charged with the task of successfully combating ready-developed purulent processes; and that almost all the known pathogenic micro-organisms—anthrax bacilli and pyococci(pus-cocci)foremost among them—readily accumulate such colorants within their own bodies, just as

larger plants do, and succumb to their toxic agency. Anthrax bacilli, pyococci, etc., as may be readily observed by the microscope, imbibe those colorants like a sponge; so that the bacteria may be noticed as being already deeply dyed before any of the colorific liquid itself becomes discernible in the field of vision; and the moment the intensive coloration is accomplished, every swarming motion ceases: the cell dies!

Although the specimens then exhibited were not allowed to be distributed, Herr Merck kindly sent me from Darmstadt some samples of the various preparations of pyoktanin which he was preparing for the market. With these, I at once began a study of its value, working along the lines already laid down in the earlier part of this paper, and with the kind advice and assistance of Dr. Behring.

Pyoktanin is furnished in two colors, *blue* and *yellow*, of which the former is much the more soluble. Of each of these a 1 to 1,000 solution was made.

In the following tables where a growth was found it is so indicated by the sign +, while the failure to grow or to develop is indicated by —.

I. Hanging drop cultures (bouillon) of anthrax, with yellow pyoktanin, at 37° C.

	1ST DAY.	3RD DAY.
Control	+	
1-7,000	+	+
1-3,500	—	+
1-1,400	—	—
1-700	—	—
1-500	—	—

II. Ditto with staphylococcus pyog. aureus.

	1ST DAY.	3RD DAY.
Control	+	
1-7,000	+	
1-3,500	+?	+
1-2,500	—	+?
1-1,400	—	—
1-700	—	—

III. Ditto with streptococcus pyogenes.

	1ST DAY.	2ND DAY.
Control	+	
1-7,000	+	
1-3,500	+	
1-2,350	+?	+
1-1,750	—	—

IV. Hanging drop cultures of anthrax, in *serum*, with yellow pyoktanin, at 37°C.

	1ST DAY.	2ND DAY.	3RD DAY.
Control	+		
1-7,000	+		
1-3,500	+		
1-2,350	?	+	
1-1,750	—	?	+
1-1,200	—	—	—

Ditto, with staphylococcus aureus (calves serum).

	1ST DAY.	2ND DAY.
Control	+	
1-7,000	+	
1-3,500	+	
1-2,350	?	+
1-1,750	—	—

These experiments were all made with the yellow pyoktanin. Similar work with the blue variety showed that it was more active, nearly doubly so, in fact. It is unnecessary to give the tables here after this statement.

Spore-thread cultures were next made, of which the following table will serve as a sample:

VI. Spore-thread cultures of anthrax (hanging drop) in bouillon, with blue pyoktanin, at 37° C.

	1ST DAY.	2ND DAY.	3RD DAY.
Control	+	Spores.	All spores.
1-80,000	+	+	
1-40,000	+	+	
1-20,000	—	+	+
1-10,000	—	—	+
1-5,000	—	—	+
1-3,500	—	—	—

Next a series of tubes of agar were impregnated with various proportions of yellow pyoktanin and cultures were attempted with the following results:

VII. Cultures of anthrax in agar, with yellow pyoktanin in proportions following, after 48 hours, at 37° C.

Control	+	
1-20,000	+	
1-10,000	+	
1-5,000	+	
1-2,500	+	Bacilli still abundant, only with relatively fewer spores.

VIII. Ditto, only with staphylococcus aureus.

Control	+
1-20,000	+
1-10,000	+
1-5,000	+
1-2,500	+

As a variation of this experiment I allowed a 1-1,000 solution to stand on top of a pure culture of staphylococcus aureus for 48 hours, then poured it off and transferred from this to a fresh tube. In 24 hours there was a luxuriant growth; showing that even 48 hours' exposure after this fashion failed to destroy this species.

IX. Next 5 cc. sterilized bouillon were inoculated with anthrax and carefully shaken. Solution of yellow pyoktanin was added till the preparation stood 1-1,400. (This was examined after 24 hours at 37° C., and no evidences of growth were found).

A. After 5 minutes' exposure a second tube was inoculated from this. In this tube *A*, after 24 hours in the thermostat, there was no growth perceptible; after 48 hours there were a few threads without spores.

B. After 2 hours a third tube was inoculated. In this, after 48 hours, there was no growth.

C. After 24 hours a fourth. In this, after 48 hours, no growth.

X. Same, except with staphyloeoccus aureus. In the original tube, 1-1,400, after 24 hours there was abundanee of zoöglœa masses.

A. (5 minutes). In 24 hours rapid growth.

B. (2 hours). In 24 hours abundance of single cocci; in 48 hours zoöglœa masses.

C. (24 hours). After 48 hours abundant growth.

XI. Same, exeept with streptococcus pyogenes.

A. In 24 hours slight growth, which after 48 hours became abundant.

B. After 24 hours nothing; after 48 hours evident growth.

C. After 48 hours nothing.

XII. Streak cultures of anthrax on agar, with yellow pyoktanin in following proportions, after 48 hours in thermostat at 37° C.

Control	Typical growth.
1-2,000	Growth, but not so rich.
1-1,000	Limited growth.
1-750	Still more restricted.
1-500	Perceptible only along the streak and in good light.
	In the condensation water in the tube bacilli appear to have grown with considerable freedom.

XIII. Ditto, but with staphylococcus aureus.

Control	Typical growth.
1-2,000	Same.
1-1,000	Same.
1-750	Limited growth.
1-500	Only slightest appearance at isolated points.
	In the condensation water cocci have multiplied, but not in abundance.

The last results noted in XII and XIII would seem to imply that the agar holds the material and that the condensation water had lost its proportion of the same. Numerous coagula or flashes in the agar were more deeply stained and may have taken up an undue proportion of the dye by selective affinity.

XIV. Experiments to determine absolute toxicity.

For this purpose a solution of 1-100 of yellow pyoktanin, since, this being weaker than the blue, if this were absolutely toxic the other could he considered more so.

a. A rabbit weighing 1200 grams received 0.03 in the abdominal cavity (in 3 cc. water). At the same time under its skin 0.03 more;—*i. e.*, in all 0.06, $=1/16$ gram. This was equivalent to one twenty-thousandth of its weight of the drug. This produced temporary toxic symptoms, from which it recoved with apparent difficulty.

b. A second rabbit of same weight received three times the same amount, say one seven-thousandth of its weight, and died in a few hours.

c. Two white mice, weighing each 15 gr., received $1/4$ cc. of 1-100 solution, in abdomen; *i. e.*, one six-thousanth of their weight, or only one-fourth of what they should receive providing they could tolerate the drug in proportion of 1-1500. One died in $1\,1/2$ hours; the second barely recovered.

d. This second mouse, three days later, received a second dose of one three-thousandth of its weight under the skin of its back; it died soon after.

e. A mouse received one three-thousandth of its weight subcutaneously. Twenty hours later, having apparently recovered, the dose was repeated, after which it soon died. Two others received, each, one six-thousandth in the back; 24 hours later one was in condition of tremor and spasm and soon died, while the other was less affected, but died after some 40 hours.

f. Another mouse received one twenty-five-thousandth of its weight, and, 20 hours, appeared recovered; then it was given a three-thousandth more, and soon died.

g. Another mouse, which received one fifteen-hundredth of its weight, subcutaneously, died very quickly.

From all of which it appears that yellow pyoktanin must be present in strength of at least 1 to 1,500 before it can be considered an antiseptic, and the solution must be even stronger than this to prove reliable. Furthermore, that in proportions in which it can be considered relatively toxic, *i. e.*, to bacteria, it is absolutely toxic to animals;—which facts relegate it to a very low position among antiseptics, and seem to disprove all claims as to its great merits. I did not long pursue my inves-

tigations concerning the blue variety, since it was quickly found that it gave scarcely any different results from methyl violet and some of the other aniline dyes, which had been already tested in the Berlin laboratory, and found not to be at all reliable when in weaker proportions than 1-3500 or thereabouts.

Moreover it has since appeared that blue pyoktanin is nothing but methyl violet free from arsenic, or chemically pure, while the yellow variety is merely one of the yellow aniline derivatives freed from deleterious admixture. The protection of these substances by trademark, and the secrecy observed on their introduction, would therefore appear to be merely a trade subterfuge.

I have been lead to detail my experiments with the material not merely as illustrative of a method, but because numerous articles have recently appeared with reference to it, in some of which the writers appear to have allowed their verdicts to be influenced by what the manufacturers have claimed for it rather than by anything like a scientific test of its genuine value.

I would not wish to be understood as inveighing against a certain well-known value which most all of these aniline preparations have in common. In 1872, Dr. Chas. Curtman, of St. Louis, made known the fact that they possess antiseptic properties, and common experience has since confirmed his statement. Stilling has gone so much further as to assert that they are absolutely non-poisonous, a statement which is far from justified by facts. Behring has pointed out the remarkable correspondence between them all, that their absolute toxicity is four or five times as great as their relative toxicity, or their antisepticity, which is corroborated by my own results given above.

In fact this is true of well nigh every antiseptic tested; and though reactions between a given substance and a particular species may show, now and then, wide variations, the general statement is beyond controversy. Indeed we see the same thing in other directions; thus (*vide supra*) anthrax bacilli will not grow on blood-serum from the rat, and Metschnikoff's vibrio can scarcely be planted in the blood of living mammals, though pigeons succumb in a day or two.

Referring back to our particular subject I would like to quote from Stilling's paper (loc. cit.) the following directions for its use in surgery and ask you to contrast them with the results of experimental tests.

The surgical antiseptic methods by means of *Pyoktanin*, I conceive to be carried out as follows: The instruments are to be simply well cleaned; or, if extra caution be desired, to be steeped, for some time preceding the operation, in a weak solution of *Pyoktanin*,—say about 1:10,000 or 1:20,000. After the operation, the wound is to be washed with a somewhat stronger solution of the same,—say 1:5000 to 1:2000. The needful stitching is to be done with silk impregnated with a 1:1000 solution of the same. Finally, the dressing of the wound would consist of antiseptic cotton and antiseptic gauze, also prepared by steeping in a 1:1000 solution of the same medicament. Thus prepared dressing materials are not only reliably *aseptic*, but also reliably *antiseptic*, and even *disinfectant;* for the slightest *secretion* of fluids within the territory of the operation must at once cause an *absorption* of a sufficiently concentrated solution of the *Pyoktanin*. Purulent developments in *puncture-channels* ought certainly not to be possible under this propbylactic *Pyoktanin* treatment.

I have no hesitation in asserting that even the blue pyoktanin —the stronger—can not be relied on in strengths above indicated for purposes claimed.

Another kind of claim was made for the material, which includes its stimulating and other desirable properties, by which it is expected to subserve useful clinical purposes. As an injection in gonorrhœa, I have had no experience with it, but find that most of those who have tried it have met with disappointment. Upon granulating surfaces it does appear to be stimulating and to exert a desirable effect, but no more so than other substances within easy or easier reach, and its stain is often undesirable. In ophthalmological practice it appears also to have scarcely come up to the requirements of the day. On the whole, then, it has but few qualities by which we are to commend it above numerous other drugs of its general class, while in all that may answer to the more scrupulous demands of aseptic surgery it has proved in my hands—as in those of others who have tested it from the purely clinical standpoint—disappointing.

Recent Literature Concerning Pyoktanin.

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